THE INTESTINAL EPITHELIAL CELL

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The Campbell Oration delivered on 2nd May, 1968

I SHOULD first like to thank you for inviting me to be the Robert Campbell Orator on this occasion. For anyone in the field of medical science it is a great honour to give this lecture, for a Queen's graduate it is a very special honour, but for a Queen's graduate who is also an exile, it is not only an honour but a very welcome opportunity to come back again to this medical school. Although my visit is a short one, I have used my time well and have taken the opportunity to see some of the great changes which have taken place since I worked here. I have been particularly impressed by what I might call the magnificent hospital campus you have got, with a wide variety of buildings and activities all within easy reach of each other. You have obviously put a great deal of careful long-term planning into the various developments of your medical school, which is going to yield rich rewards in the days to come. By some standards Queen's is a relatively young medical school, but your well-planned broad acres for development must be the envy of some of the older medical schools whose traditions are far more extensive than their building space.

The Robert Campbell Oration commemorates a great individual in Ulster medicine, but in the present context the commemoration might be extended to include not only Robert Campbell himself but others who bore his family name. I am very proud to be able to claim a fairly close contact with that name, for the late Mr. W. S. Campbell and I were exact contemporaries. We came up from school together to study medicine at Queen's. After the 2nd M.B. examination we both took time off for an honours B.Sc. in Physiology. We completed our medical course together and went to house posts at the Royal Victoria Hospital. We came back to Queen's again and spent three years as demonstrators, he in Anatomy, and I in Physiology. Knowing Bill Campbell at that time probably better than anyone else here, I should like to take a moment to pay a tribute to the brilliance of an undergraduate career, which I am sure has never been surpassed and probably never equalled. From the second year onwards he won every honour the medical school had to offer, every prize, medal and scholarship which was available. He did this with such a characteristic modesty, and with such an absence of any kind of consciousness of his own great abilities that we all entirely accepted him as first without any feeling of envy, and we were happy to compete amongst each other to be second to him. His undergraduate career was indeed worthy of the name which we are honouring this evening.

I should like to talk to you about the intestinal epithelial cell, and in doing so I shall stress a particular approach to the problem of cellular function which I and my colleagues in Sheffield have been using and which we have called 'functional topography'[1]. Let me explain first what we mean by this term. When we think about how any part of the body works we are conditioned to think in terms of structure and function in that order. It starts from our earliest training, for most of us had our first introduction to medical studies in the dissecting room, and we

learned to think about the structure of the body before we considered its functions. In the macroscopic field this has perhaps some validity for we must know something about the structure of things whose functions we are going to investigate. In the microscopic field, however, I am less certain that this principle holds. The immense developments in microscopy over the last few decades have increased greatly our capacity to see the structures in the cell, even to the level of locating the enzymes, but this in itself leaves a good deal unknown about cellular function. In the approach I wish to describe, we begin not by thinking about the structure of the cell but rather by trying to find out some of its functions and to plot these relative to each other. Topography means making a map of a region to delineate the features of it. In our topography of the cell the features are the functions not the structures, and hence we call this functional topography. The object is not to ignore the enormous amount of knowledge on cell structure, but to supplement this with parallel studies on the topography of function. Instead of asking what functions can be assigned to particular structures the microscopist has discovered, we would reverse the question and ask what structures could serve as sites for functions which can be identified in the cell, and at least in a very rough way related spatially to each other. I am not attempting to give an extensive review of the intestinal cell, but to present a type of approach, and the references therefore chiefly relate to work carried out in Sheffield. I should like to acknowledge here the great help I have received from numerous colleagues in Sheffield and in particular Drs. R. J. C. Barry, H. Newey and P. A. Sanford.

The intestinal epithelial cells absorb all the food which we need, and a good many of the drugs which we think we need. It is now well recognised that there are diseases of the cell, which form the malabsorption syndrome, in which the patient suffers because the epithelial cell is not functioning normally. As distinct from this medical interest in the epithelial cell, there is the interest of the biologist. The biologist is concerned in knowing how cells work and he seizes on any cell, if if it throws light on the general problem of cellular function. It so happens that the intestinal epithelial cell has great attractions from this point of view. It acts in a very special way as a transducer, if we may borrow the term from the engineer. A transducer is an instrument which changes energy from one form into another, and the special feature of the columar cell is that it changes chemical energy into osmotic work. Furthermore, it does this in a position which makes it very accessible to experimental investigation, and few cells in the body offer such an attraction. While there are these two approaches to the cell, the medical one and the biological one, it might be thought that to a medical audience I would have chosen the one of medical interest. Paradoxically perhaps, I have chosen not to do so, and this is because I regard my audience not only as individuals who are employed in providing a health service to the community, important though that may be, but also as biological scientists with a unique opportunity for observing the most interesting and complex of all biological creatures, the human being himself. I therefore make no apology for omitting the medical interests of the intestine and concentrating on how the intestinal epithelial cell works, with a view to throwing light on the whole problem of cell activity and how the energy of metabolism is used by the cell for its various purposes. When we can answer these problems the medical aspects of cell function will perhaps become clearer.

Let us begin by looking at a simplified diagram of the intestine in order to see the general orientation of our problem (Fig. 1). The essential part of the intestine which interests us is the layer of epithelial cells, which is in contact with the luminal contents, and which consists of two kinds of cells – columnar cells and goblet cells. The diagram omits the goblet cells, which secrete mucus and are not related to absorptive activities. The columnar cells transfer substances from the lumen of the intestine into the subepithelial fluid. From there they pass into the blood or

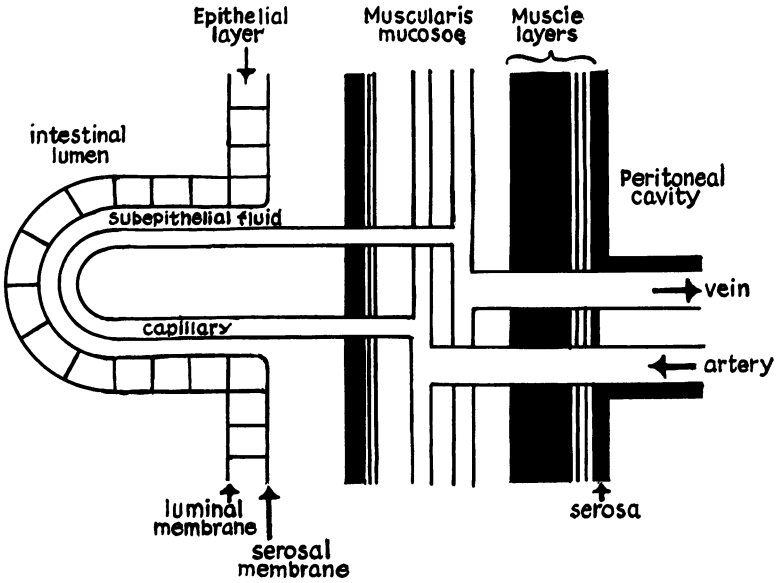


FIG. 1. Diagrammatic representation of the gut wall to show the essential structures involved in absorption (from Principles of Human Physiology. Ed. H. Davson & G. Eggleton. London: Churchill. In Press.)

lymph vessels and are removed. The process of transfer from subepithelial fluid to the blood or lymph vessels are relatively simple ones depending on the pores in the capillary walls. There is no reason to think that any more complex processes are involved, and the basic activity of absorption, i.e., the selectivity, the carrying out of osmotic work, the utilisation of metabolic energy, reside in the columnar cell, and it is on these we will concentrate.

Our experiments consist fundamentally of observing changes in the fluid on the two sides of the cell. On one side – the luminal side – there is no difficulty. The luminal fluid is readily accessible to experimental investigation. The other side – the subepithelial fluid is less accessible, and presents much more difficult problems. As substances pass into the subepithelial space, they do not remain there, but diffuse into the capillaries and are carried away. Furthermore, collection and examination of the mesenteric blood do not supply an adequate answer to the problem. For one thing they introduce enormous complexity due to the presence of the other blood constituents – particularly the proteins. But more important the rate of mesenteric blood flow means that absorption of even large amounts of

substances only cause small changes in blood composition. We must therefore look for some other method of studying the epithelial cells, even if it involves conditions different from the physiological ones.

For long it has been known that many organs can function for a short time separated from the body and with saline as a nutrient medium – e.g. heart, muscle, nerve, etc. The intestine too has been used in this way, but until recently only to study the properties of the smooth muscle in the intestinal wall. Attempts from time to time to use the isolated intestine to study absorption were all unsuccessful, until Fisher and Parsons[2] found that the intestine could function provided certain precautions were taken, and essentially these were to provide an oxygen supply to the epithelial cells. The solution of the problem was to circulate oxygenated fluid through the lumen of the intestine as illustrated in Fig. 2, and in these conditions it was found that the intestine could transfer certain substances from the fluid in

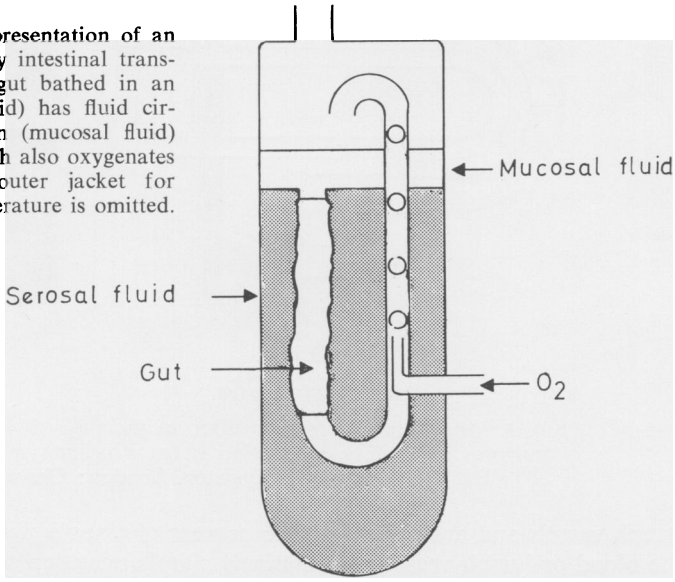


FIG. 2. Diagrammatic representation of an *in vitro* technique to study intestinal transfer. A small segment of gut bathed in an outer solution (serosal fluid) has fluid circulated through the lumen (mucosal fluid) by means of an 0_2 lift which also oxygenates the mucosal fluid. An outer jacket for maintaining constant temperature is omitted.

contact with the mucosa (the mucosal fluid) to that in contact with the serosa (the serosal fluid). A very important development of this technique was introduced in Sheffield by Wilson and Wiseman[3] who turned the intestine inside out and made the everted sac, and these *in vitro* techniques have since been modified and exploited extensively by many subsequent workers[4, 5, 6, 7]. (The term *in vitro* is applied to all intestinal preparations deprived of their blood supply, while *in vivo* means that the intestine is in situ in the living animal, or at least is receiving a blood supply through the mesenteric vessels). The success of the *in vitro* procedures is largely in their amazing simplicity, and the everted sac can readily be used as a class experiment. The essentials of the technique are seen in Fig. 3. A small animal must be used because the wall of the intestine must be fairly thin, and rat, hamster and guinea-pig are most frequently employed. A piece of intestine is everted, and then made into a sac, by tying one end, filling it with saline and then tying the other. The sac is put into a flask containing saline, the saline is oxygenated and the

flask shaken at 37° C. During this process substances are transferred from the outer fluid in which the sac is shaken (the mucosal fluid) into the fluid inside the sac (the serosal fluid). While this capacity for transfer can be readily demonstrated it is pertinent to ask its relation to physiological absorption[8]. In

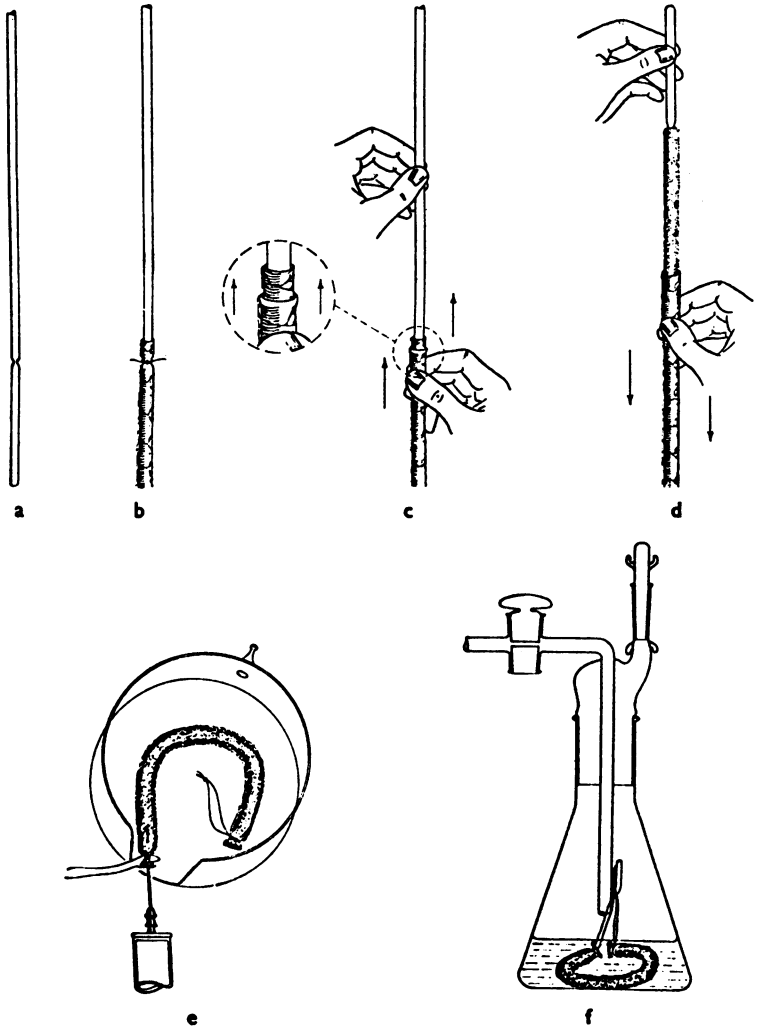


FIG. 3. Some stages in making sacs of everted small intestine for absorption studies. *Above:* Eversion of the intestine. A glass rod is used with a groove provided for a ligature (a). This is inserted into the intestine which is then tied close to the end (b). The eversion is commenced by pushing the gut upwards over the place where it is tied (c). The eversion is completed by inverting the rod and pulling the intestine downwards (d). *Below:* (e) Filling the sac, which is lying in a specially modified Petri dish used for weighing the gut. *Below:* (f) The sac in the incubation flask, which is provided with arrangements for gassing the flasks with O_2 or an O_2 and CO_2 mixture. The glass hook enables the sac to be lowered into the flask and to be removed again at the end of the experiment. (Drawing by K. Curtis), from Recent Advances in Physiology, Ed. R. Creese, Churchill: London, 1963.

physiological absorption the substances pass into the blood stream. In the *in vitro* intestine they pass through the whole layer of the wall of the intestine. The activities *in vivo* and *in vitro* are not however as different as might be thought at first sight. Physiologically substances are transferred by the epithelial cells into the sub-

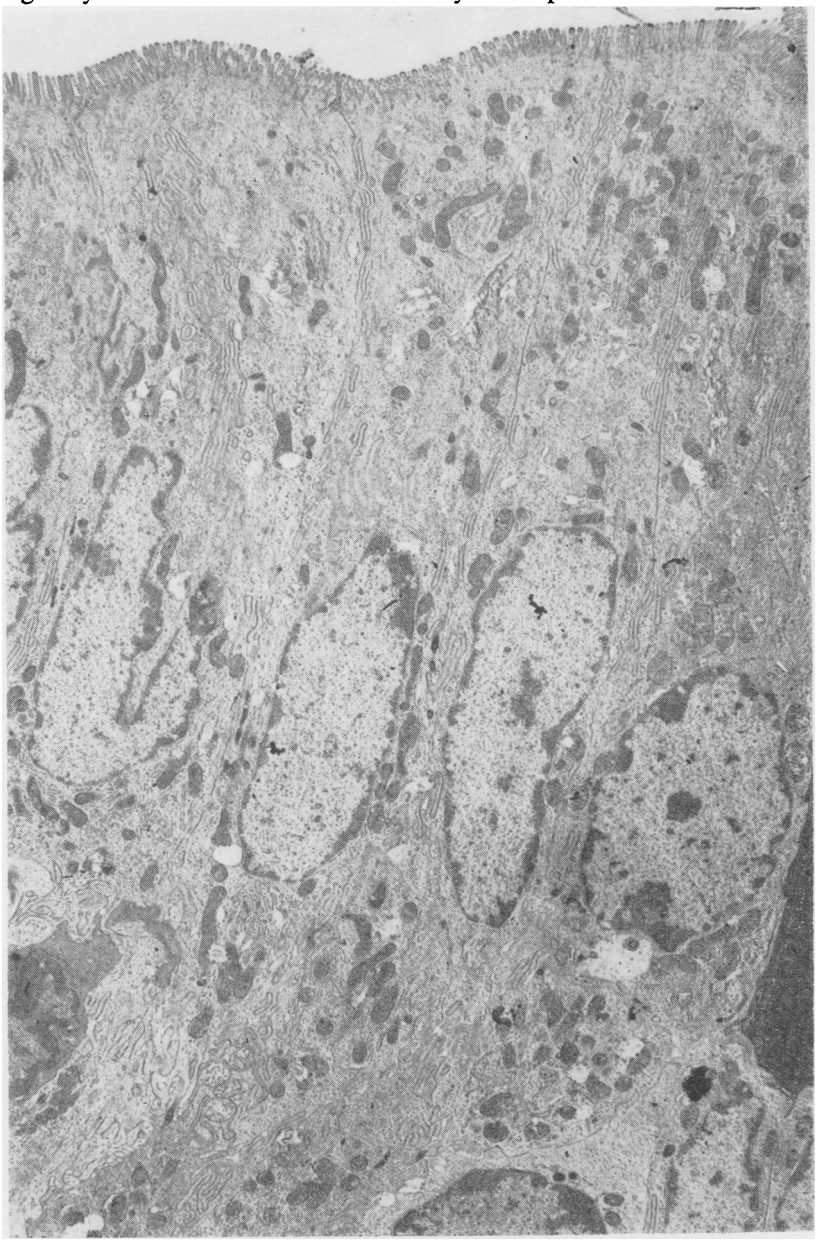


FIG. 4. Electronmicrograph of epithelial cells from rat small intestine (x 5,000). By courtesy of Mr. J. H. Kugler.

epithelial space, and then removed by the blood flowing through the capillaries. In the *in vitro* preparation the same process of transfer by the epithelial cells occurs, and the only difference is the method of removal from the subepithelial space. There is now no blood flow, and the substances escape either by diffusing through the muscle layers or by passing out through the cut ends of the blood vessels. There is good evidence that this latter process occurs, for the first fluid to be transferred is blood stained. Furthermore, a very large number of experiments have been done to compare the activities of *in vitro* preparations with the intestine in normal physiological conditions and there is an impressive body of evidence that these are very similar. There are certain differences, but these are chiefly related to the supply of metabolisable substrate to the epithelial cell, and these differences can be exploited[9] to study the relation between energy and transfer processes, a subject referred to later.

Our object was to deduce what was happening in the cell from the changes in the fluid on both sides. As we have seen this is not strictly possible, but by studying the changes in the fluid on the two sides of the gut wall, instead of on the two sides of the epithelial cell, we can approximate to the desired condition. In the experiments described here we speak of the mucosal and serosal fluids and the diagrams are simplified by ignoring the other layers of the gut wall. This oversimplification for purposes of discussing a fairly complex subject in a limited time does not mean that the difficulties have been ignored or that the limitations of the technique are not appreciated.

Let us now look at the orthodox histological structure of the epithelial cell as presented in Fig. 4, which shows the various features of the cell, the brush border in contact with the intestinal lumen, the other structures to which a variety of

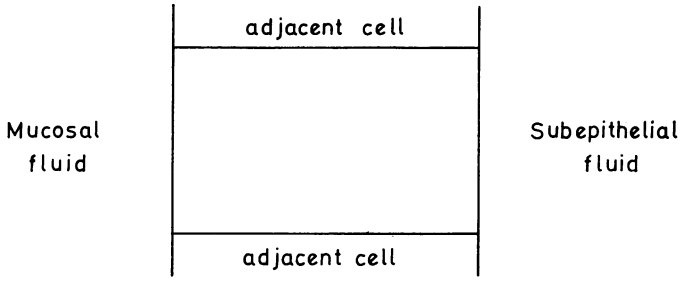


FIG. 5. Diagrammatic outline of the epithelial cell in which are defined only (a) surface in contact with the mucosal or luminal fluid, (b) surface in contact with the subepithelial fluid, and (c) surfaces in contact with adjacent cells. In all subsequent diagrams of cells it is assumed that the cell has the same orientation as shown here with regard to mucosal and subepithelial fluid.

names have been given – the terminal bar, the terminal web, the mitochondria etc. Now instead of trying to discuss what the functions of all these structures are, our functional topography begins in an entirely different way by representing the cell as a blank rectangle and attempting to map the location of the different activities (Fig. 5). The only features we label at the moment are the mucosal and serosal

surfaces, and the part in contact with adjacent cells. In practice the cell is not rectangular, but for our purposes this suffices, provided we define the mucosal side as that part in contact with the luminal contents, while the serosal side is the part in contact with the subepithelial fluid.

We begin with an experiment in which we begin with glucose on each side of the cell in the same concentration of 28mM (Fig. 6). At the end of an hour the glucose

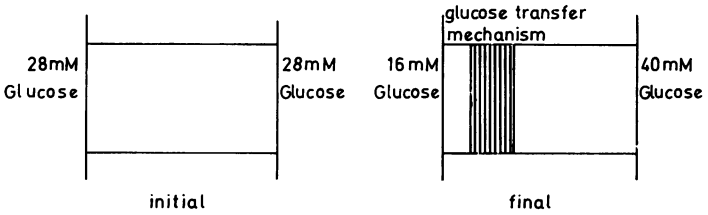
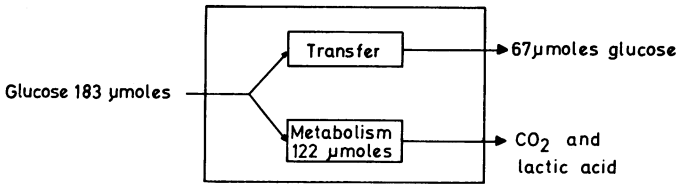


FIG. 6. Experiment showing moving of glucose against a concentration gradient, and hence need to locate in the cell a glucose transfer mechanism.

concentration in the two fluids is measured and is found to have fallen on the mucosal side and risen on the serosal side. We therefore say that the cell has got a mechanism for transferring glucose from one side to the other and we put into the cell our first function, a glucose transfer mechanism, or in the jargon frequently used now in discussing cell transport, a glucose pump. This picture can quickly be recognised as an oversimplification by including not only the concentration of glucose but the amounts involved (Fig. 7). We start off with 416μmoles glucose.

	μmoles	
Total initial glucose		416
Mucosal transfer	183	
Serosal transfer	67	
Glucose in gut wall	44	
Glucose recovered		294
Glucose metabolised		122

FIG. 7. Balance sheet of the amounts of glucose initially present, the final distribution and the calculated amount metabolished. As a result a metabolic compartment is postulated, in addition to a transfer mechanism.



We find that 183μmoles disappears from the mucosal side (we refer to this as the mucosal transfer). Of this 67μmoles appear on the serosal side (which we call the serosal transfer). We recover 44μmoles from the gut wall. If we add these together we have recovered 294μmoles of glucose so that there is 122μmoles unaccounted for, and we must assume that this has been metabolised. We therefore revise our picture of the cell and as shown in Fig. 7 the missing glucose appears as lactic acid and CO_2. The lactic acid is not simply an artifact due to the poor condition of the cells, but it is known that even *in vivo* conditions[10] much of the metabolised glucose appears as lactic acid. We therefore place in the cell two processes for dealing with glucose, one transfer and one metabolism.

Let us think of this metabolism a little more closely. In Fig. 7, glucose came from the luminal side of the cell, but physiologically glucose would also come from the other side of the cell, because like all tissues the cell would receive nutrition from the blood stream. We therefore have to assume that the epithelial cell can get glucose from both sides, from the lumen of the intestine and from the blood stream. This condition can be simulated *in vitro* by putting glucose into the serosal fluid also.. Furthermore, we can find out which side of the cell uses glucose by using ^{14}C labelled glucose on one side and ordinary glucose on the other[11]. Analysis of the specific activity of the carbon dioxide shows that glucose from both sides is metabolised, but that rather more glucose is metabolised from the mucosal side than from the serosal side.

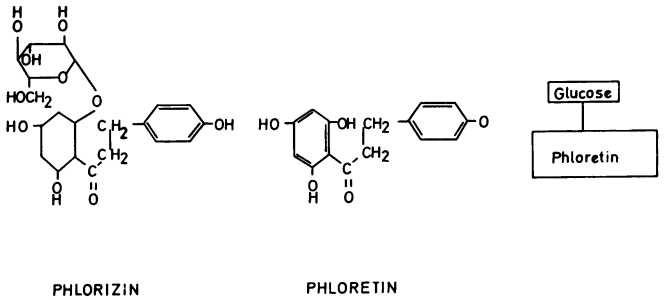


FIG. 8. Structural formula of phlorrhizin and phoretin. As indicated at the right hand side, phlorrhizin may be represented as a large phloretin molecule with a glucose molecule attached.

We now make use of a substance which for a long time has been used in the study of carbohydrate metabolism in the body, namely phlorrhizin. Many years ago it was found that if phlorrhizin was injected into the animal glucose appeared in the urine, the reason being that phlorrhizin prevented the reabsorption of glucose in the renal tubules. A long time afterwards it was found that phlorrhizin also prevented absorption of glucose from the intestine[12], and this activity can be used to throw light on the mechanism for dealing with glucose. The structural formula of phlorrhizin is shown in Fig. 8, and can be regarded as consisting of two parts, a large molecule of phloretin and attached to this a molecule of glucose. The importance of glucose will be apparent later. An experiment can readily be carried out similar to that shown in Fig. 6, but in the presence of 5 x 10^{-4}M phlorrhizin in both

the mucosal and serosal fluid and Fig. 9 shows the results of one such experiment, and illustrates the dramatic effect of phlorrhizin in inhibiting glucose transfer. No glucose disappears from the mucosal side and the concentration on the serosal side does not rise but actually falls. (This fall is due to glucose diffusing from the serosal fluid into the gut wall, and need not concern us further here). It can be assumed then that some part of the transfer process is phlorrhizin-sensitive. This can be investigated further by examining the metabolism of glucose in the presence

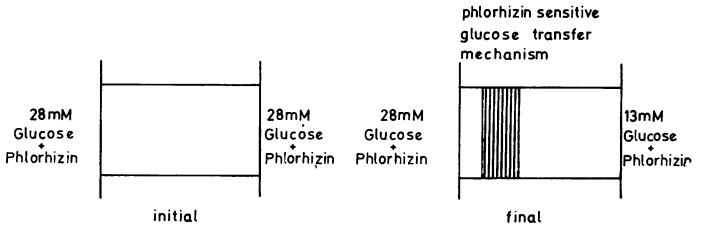


FIG. 9. Experiment showing the effect of phlorhizin (5×10^{-4}M) in inhibiting glucose transfer, and hence postulation of a phlorrhizin-sensitive transfer mechanism.

of phlorrhizin and this is shown in Fig. 10. In these experiments ^{14}C glucose is used in the mucosal fluid and unlabelled glucose in the serosal fluid[11], and the experiments are done in the presence and absence of phlorrhizin. In the absence of phlorrhizin glucose is metabolised from both sides, so that the CO_2 from metabolism is partly labelled. In the presence of phlorrhizin no labelled CO_2 appears. From this it can be concluded that phlorrhizin prevents the entry of

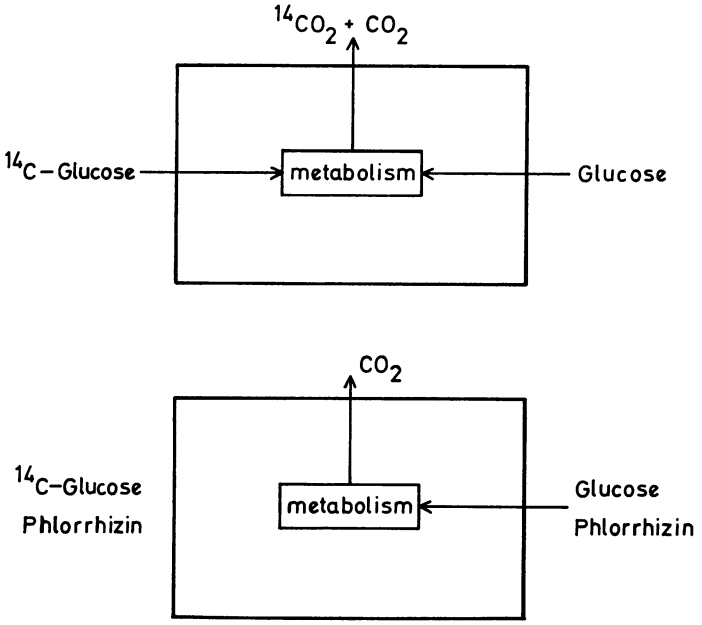


FIG. 10. Experiment to show the effect of phlorhizin on the metabolism of glucose initially present in the mucosal or serosal fluid.

glucose from the luminal side of the cell but does not prevent entry from the serosal side. In other words we have shown clearly that the cell is not symmetrical with regard to phlorrhizin. One side is different from the other and entry of glucose from the mucosal side depends on some mechanism which is sensitive to phlorrhizin. We therefore put into the cell a mechanism which is phlorrhizin-sensitive and which must lie between the lumen of the intestine and the site of glucose metabolism (Fig. 11). We are thus beginning the process of localisation of functions in relation to each other.

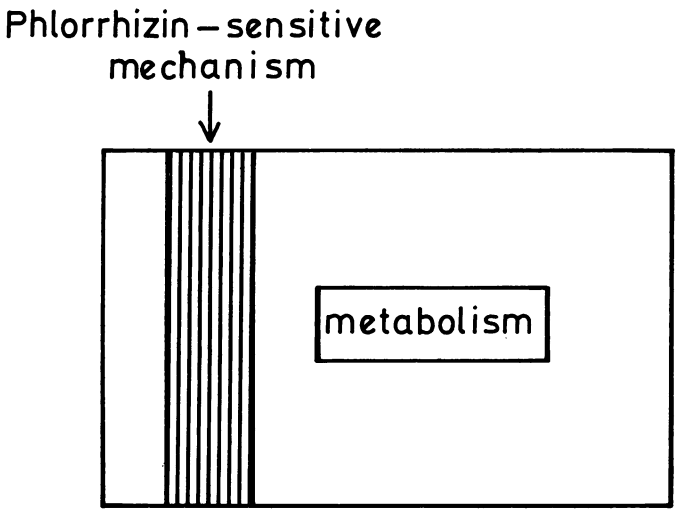


FIG. 11. Location of a phlorrhizin - sensitive mechanism between the luminal border of the cell and the site of metabolism.

So far only glucose has been considered in this discussion. Glucose forms only a small part of the carbohydrate intake, which is mainly in the form of starch. This is broken down by amylase, present in saliva and pancreatic juice, to maltose and the disaccharide is then broken down to glucose by the enzyme maltase. It was long believed that maltase was present in the succus entericus, which represented a digestive secretion analogous to gastric or pancreatic juice. This view of the succus entericus has been questioned from time to time since Starling[13] showed that if the juice is centrifuged, it consists of a clear supernatant and a deposit and the clear supernatant was much less active enzymatically. It therefore follows that the maltase activity is present mainly in the deposit. We know that this deposit consists of shed epithelial cells and the maltase must be present in these dead cells which have been shed off into the lumen of the intestine. It is easy to argue one step further[14]. If the maltose can penetrate into these dead cells and get hydrolysed, might it not also penetrate into the living cells lining the gut. In other words might some of the maltose activity not occur intracellularly? This can easily be tested experimentally, and such an experiment is illustrated in Fig. 12.

We make two identical sacs of everted intestine and incubate each of them in saline for 60 minutes in a small conical flash[15]. In one case 675μmoles of maltose is present in the saline and in the other case no maltose is present. At the end of 60 minutes we remove the two sacs and in the maltose experiment we estimate the

amount of glucose present and find 672μmoles. This must have been due to maltase activity which could have been present either in the epithelial cells or could have been secreted into the surrounding fluid. In order to show which, we now add

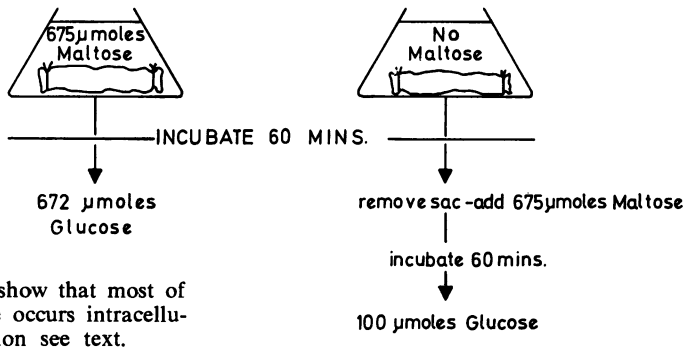


FIG. 12. Experiment to show that most of the hydrolysis of maltose occurs intracellularly. For explanation see text.

675μmoles of maltose to the fluid in which the sac was incubated without maltose, and we incubate a further 60 minutes. We find that only 100μmoles of glucose is present. This experiment shows quite decisively that the hydrolysis of maltose must have taken place mainly in the epithelial cells of the intestine and was not due to maltase which has passed into the surrounding fluid. We therefore must place in

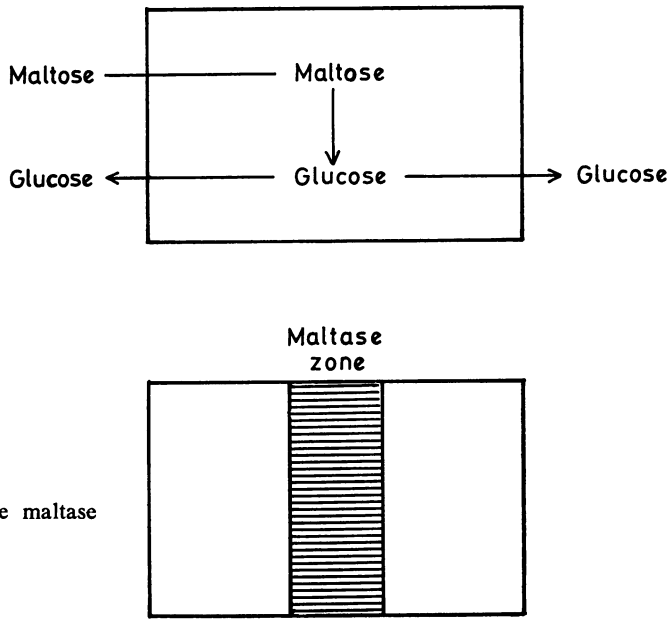


FIG. 13. Postulation of the maltase zone in the cell.

the cell a maltase zone (Fig. 13) and for the moment we insert this without relation to the other cellular functions. (This concept of intracellular hydrolysis was in fact first used in relation to peptide digestion [16] and later applied to disaccharides[15 & 17].

Having decided there is maltase activity in the cell, can we localise this in relation to the topography of Fig. 11. This in fact can be done by use of phlorrhizin[15]. An experiment was carried out with the everted sac of intestine in which maltose was initially present in the serosal fluid (Fig. 14). At the end of an hour we find that 15μmoles of glucose is present in the mucosal fluid and 16μmoles is present in the serosal fluid. Some glucose must also be present in the gut wall and some must also have been metabolised. It is thus clear that maltose initially present

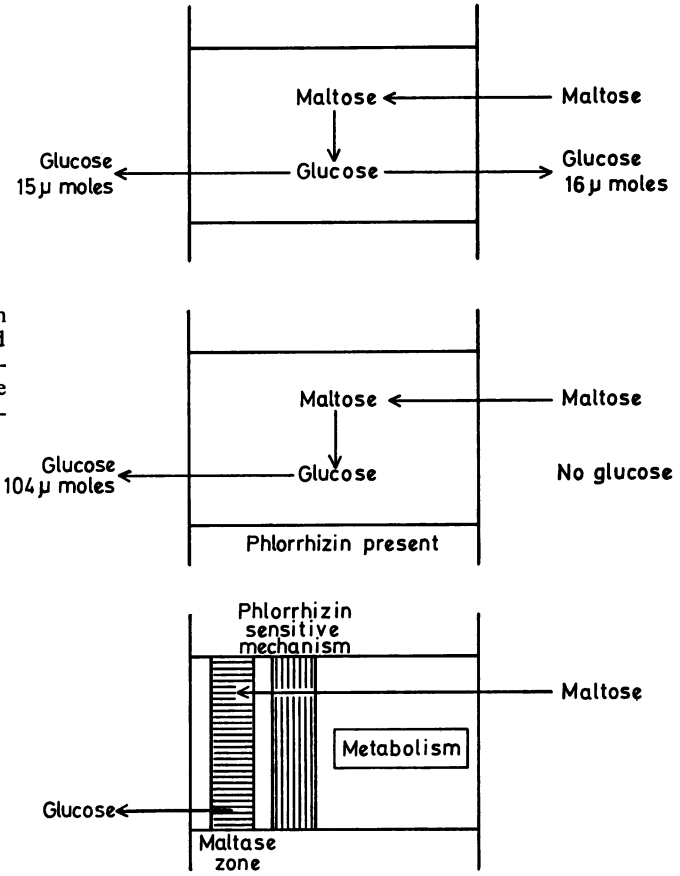


FIG. 14. Relative location of maltase zone and phlorrhizin-sensitive mechanisms by use of maltose and phlorrhizin. For explanation see Text.

in the serosal fluid can reach the maltase zone. The experiment is repeated but in the presence of phlorrhizin. It is now found that 104μmoles of glucose appeared in the mucosal fluid but none was present in the serosal fluid. Since glucose was formed maltose was still able to get into contact with the maltase zone. But it is clear that none of the glucose formed gets into the serosal fluid and furthermore, since much more passes into the mucosal fluid, it is probably also prevented from being metabolised. The only explanation for this must be that phlorrhizin-sensitive mechanism lies inside the maltase zone and we can therefore extend our topography to the picture shown in Fig. 14.

Let us now concentrate on this phlorrhizin-sensitive mechanism for entry of glucose into the cell. All cells are bounded by a membrane which is partly lipid, and some such membrane must separate the luminal contents from the inside of the epithelial cell. Substances which enter the cell must pass through the membrane, and the question arises how a hydrophilic substance like glucose diffuses through the lipid barrier. The simplest assumption would be the existence of aqueous pores in this membrane, i.e., pores filled with water, through which glucose is able to pass. The microscopical approach does not help us, as such pores could be too small for the degree of resolution even of the electron microscope. Again we use the functional approach. If pore size regulates entry, we should expect a relation between ease of entry and molecular weight. In fact, many substances have been tested[18], and the rates of entry studied. If we consider a few of these, glucose and galactose are readily absorbed, while mannose and sorbose are not, and yet all of these have the same molcular weight and roughly the same shape of molecule. This rules out the possibility of regulation of entry by pore size. It is in fact possible to get some idea of pore size expressed by the equivalent pore radius[19], and a simple method of determining this in the intestine[20], has shown that the pore size is too small to permit passage of glucose.

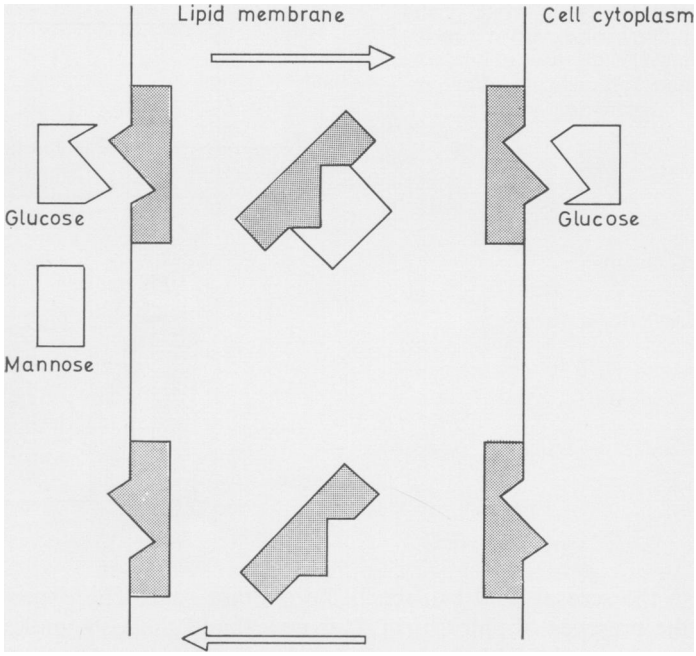


FIG. 15. The carrier concept in membrane transfer. The carriers are the shaded structures in the lipid membrane, which can cross the membrane either with attached molecule (glucose) or unattached. The specificity of the carrier is indicated by the geometric configuration which fits the molecule carried e.g. glucose but not mannose.

In order to explain the entry of substances in the cells through a lipid membrane the concept has been used of carriers, and this concept is useful not only because it explains how hydrophilic substances pass the lipid barrier, but also the high degree of chemical specificity in the process. The carrier concept has been extensively reviewed[21, 22, 23], and only a brief outline will be given here. We assume that there are substances in the membrane called carriers (Fig. 15). These have

the property of being lipid soluble so they can diffuse freely in the membrane but remain in the membrane and do not leave it. The carriers have also got specific sites to which other substances can have attached. These are analogous to the active centres of enzymes, which can give a high degree of specificity for particular subtrates. A number of carriers have been postulated in the intestine, and one of these can handle glucose. It is further assumed that when glucose is attached to the carrier the whole complex remains lipid soluble. When the carrier complex gets to the other side of the membrane glucose is set free, and the empty carrier can diffuse back across the membrane to take up more glucose. This carrier concept is however hypothetical. The carriers have never been identified or isolated, and their existence must depend on functional evidence. What sort of evidence exists for them? In the first place it might be possible to define chemically what substances are able to attach to the carrier, and it would be reasonable to expect a common structure in substances which can use the carrier. This has been done[18], and it is known that the glucose carrier also handles all substances with the basic

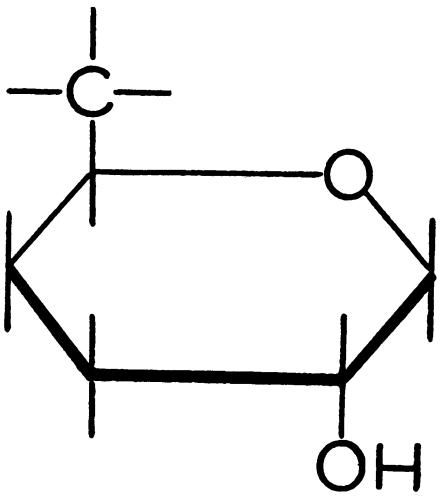


FIG. 16. Basic structure of hexoses which can be actively transferred by the intestine. From Crane, R. K. (1960). *Physiol. Rev.*, **40**, 789

structures shown in Fig. 16, i.e. a pyranose ring with a C attached to C_5, and the OH attached to C_2 in the same stereochemical position as in D-glucose. Another consequence should follow from this. Glucose and galactose both share the structure shown in Fig. 16, and can therefore use the same carrier. We might expect that if present together they might compete with each other for the carrier, and hence for intestinal transfer. This also happens. It was shown many years ago[24] in the whole animal, and more recently[25], in the *in vitro* intestine (incidentally a useful demonstration of the validity of the *in vitro* approach). Such experiments involving competition between two substances also show that while two different substances can use the site, one of them may be preferred, and in fact glucose has more effect in inhibiting galactose, than galactose has in inhibiting glucose. We say that the site has a greater affinity for glucose.

Another useful approach to the problem can be made with phlorrhizin. The formula of phlorrhizin (Fig. 8) shows that it contains a glucose residue. It there-

fore seems possible that phlorrhizin might act by this glucose residue attaching itself to the glucose site on the carrier. Furthermore, the affinity might be increased by the attachment of the lipid soluble phloretin, so that it attaches very firmly and prevents transfer of any sugars. It seemed to us[26] that there was one way in which

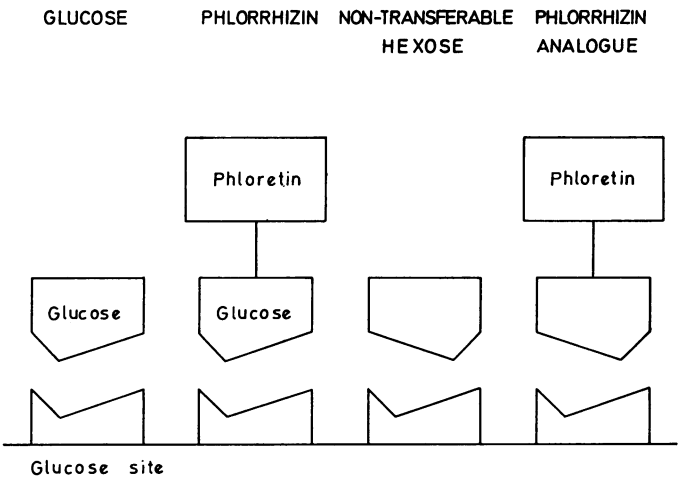


FIG. 17. Diagram to illustrate how phlorrhizin (with a glucose residue) could block glucose transfer, while a phlorrhizin analogue (with a different hexose residue) could fail to do so. The specificity of the carrier is indicated by the geometric configuration which fits glucose but not the other hexose residue.

this could be tested quite conclusively, and this is illustrated in Fig. 17. This shows the idea of phlorrhizin attaching to the glucose site, by means of the glucose residue it contains. Now supposing we could take another sugar which could not be transferred by the intestine and did therefore not fit the carrier site, and attach this to phloretin. We would then have a substance which was very similar to phlorrhizin and different only in the structure of the sugar residue. If our theory is right this phlorrhizin analogue should not be able to inhibit glucose transfer. All we required to test the theory was the suitable analogue of phlorrhizin. This however proved very elusive. It was not available commercially, and we were unsuccessful in persuading anyone to undertake the synthesis of the very small amount we required. The project was put aside until a solution turned up, and this in fact did happen from a very unexpected quarter. A chance meeting with Dr. A. H. Williams of the Long Ashton Research Station at Bristol revealed that he was classifying varieties of apple from the chemical substances which were present in the plant, and the substances he was using were different analogues of phlorrhizin. Among those he had already isolated was a rhamnoside – a phlorrhizin analogue containing rhamnose instead of glucose. Rhamnose does not have the basic structure shown in Fig. 16, it is not transferred by the intestine, and the rhamnoside was therefore the substance which we were looking for. Experiments were quickly carried out to test the effect of the rhamnoside on intestinal transfer of glucose, and these are seen in Table 1. It is evident that the rhamnoside has very little effect on glucose compared with that of phlorrhizin. These experiments supported the view of glucose entry by specific carriers.

Fig. 14 shows the picture we have built up of the intracellular topography of some of the functions. So far, however, we have said nothing of the relationship of

TABLE I

Effect of phlorrhizin analogues on absorption of glucose

Phlorrhizin or analogue	*Amount of glucose absorbed µmoles*	*Percentage inhibition*
None	250	—
Phlorrhizin 5 x 10^{-5}M	89	64
Phlorrhizin 5 x 10^{-4}M	16	94
Rhamnoside 5 x 10^{-4}M	244	2

metabolism to transfer, apart from the statement that the cellular machinery is able to convert metabolic energy into osmotic work. As an example we may consider transfer of hexose, which must be carried out at the expense of metabolic energy. It is difficult to study this problem with glucose, because it partakes in both activities – it is both transferred and metabolised. If, however, we use a sugar which can be transferred but not metabolised then we might throw some light on this problem. Galactose is such a sugar, for it is not metabolised appreciably by rat intestine, but it can be transferred against a concentration gradient. The energy for this process must come from the cell, and we assume it comes from the endogenous metabolism which takes place largely through the citric acid cycle – a process located in the mitochondrial compartment of the cell. We can represent this process

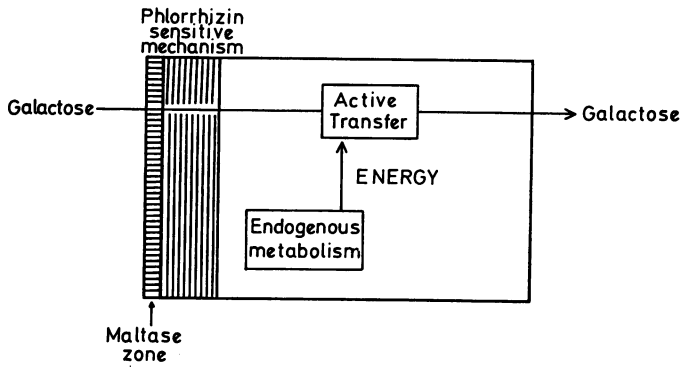


FIG. 18. Diagram to illustrate the endogenous source of energy for the galactose transfer mechanism.

by Fig. 18. Galactose enters by a phlorrhizin sensitive mechanism, and the energy for this is provided by endogenous metabolism. Previous experiments[9] had led us to believe that the supply of energy in the cell for transfer processes was limited, and it therefore seemed possible that we could increase the metabolism of the cell and hence the transfer of galactose by addition of glucose. If we put glucose in the mucosal fluid with galactose, there is a competition for the entry mechanism so that glucose will keep galactose from coming in. However, we can get around the difficulty by putting glucose on the other side, so that glucose can get into the cell without competing with galactose for the entry mechanism. The expectation would be that if glucose and galactose are present together in the mucosal fluid, galactose transfer would be inhibited, but that if galactose is present in the mucosal fluid

TABLE II

Effect of glucose in the mucosal or serosal fluid on the transfer of galactose

Initial condition		*Galactose transferred*
Mucosal fluid	*Serosal fluid*	*μmoles*
Galactose	—	117
Galactose Glucose	—	85
Galactose	Glucose	247

and glucose in the serosal, galactose transfer should be stimulated. Table II shows the results of such an experiment[27], which conforms entirely to the prediction. We thus add another complication to our cell, by putting in another metabolic com-

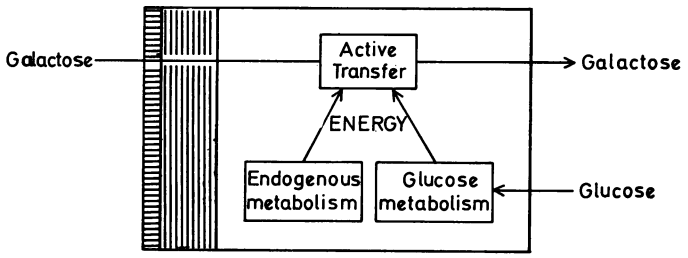


FIG. 19. Diagram illustrating the two sources of energy for galactose transfer, (a) from endogenous metabolism and (b) from glucose metabolism.

partment for glucose (Fig. 19). We know in fact that most of the glucose metabolised does not go through the citric acid cycle, but by the glycolytic pathway, and therefor we make two separate metabolic compartments.

At the beginning of the lecture, I said that if we made functional maps of the cell we might be able to assign these functions to definite structures. Is there any evidence that this can be done? Crane and his colleagues[28] have made a preparation of brush border of the epithelial cell and shown that this contains most of the maltase activity of the cell and our functional studies agree with this in placing the maltase zone close to the luminal border of the cell. Furthermore, histochemical evidence indicates that the enzymes for the citric acid cycle are in the mitochondria, while those for glycolysis are in the cytoplasm, and this agrees with the representation of separate metabolic compartments for endogenous metabolism and metabolism of added glucose.

I have run out of time, and have still only talked of one aspect of the functional topography of the cell, i.e. hexose transfer. There are many other aspects which can be studied in a similar way – amino acids[29, 30, 31], fatty acid and glycerides[32, 33], fluid and inorganic salts[34], and in each case we can make at least tentative maps of some of the processes involved in the intracellular transport. I hope however, I have achieved my main object which was not to give you an immense amount of detailed

information, but rather to illustrate one approach to the problem of cellular function.

In conclusion, could I remind you of the old biblical scholar who once said that the book we call the Acts of the Apostles was misnamed. It should have been "Some Acts of some of the Apostles". Perhaps the title of my lecture should have been "Some functions of some parts of some intestinal epithelial cells".

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